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(54) Title: A TWO COMPONENT FIBRIN-GLUE COMPOSITION FOR IMPROVING IN VITRO FERTILIZATION

(57) Abstract

A two component fibrin-glue composition comprising the components A and B, wherein component A comprises fibrinogen and a protease inhibitor, component B comprises a proteolytic enzyme being capable of cleaving specifically fibrinogen and causing formation of the fibrin polymer and additionally ingredients for culturing embryonic cells of mammals in one or both of the components A and B.

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"A two component fibrin-glue composition for improving in vitro fertilization

This invention relates to a two component fibrin-glue composition comprising the components A and B for improving in vitro fertilization.

In 1987 the first in vitro fertilization (IVF) baby was born in England. Thereafter, the in vitro fertilization became widely used throughout the world. Indications for in vitro fertilization include virtually any form of infertility.

Since the introduction of IVF the techniques have improved in all aspects of this method, for example, induction of ovulation, ovum recovery and so on except in one field where are still severe problems and complications which is the field of implantation of the embryo into the uterus. The incidence of implantation of the embryo inside the uterus is still 8 to 9 % per embryo or 20 to 30 % rate of pregnencies (since 3 to 4 embryos are normally transferred in each in vitro fertilization). It can be assumed that the transfer of young embryos (2 to 8 cells) to the uterus may play an important role in this failure to get higher rate of implantations. These embryos are not mature enough for

implantation at this stage. On the other hand culturing the embryos outside the uterus for longer time is damaging the embryos and only about 30 % of them will develop to the blastocyte stadium which is the developmental stage at which normal implantation occurs. Attempts for improvement of the implantation using a fibrin-glue known in the art failed. The idea was to attach the embryo with this fibrin-glue on the walls of the uterus (endometrium).

It is an object of this invention to provide a composition and a method for improving in vitro fertilization.

According to the invention the above mentioned problem can be solved with a two component fibrin-glue composition comprising components A and B wherein

- component A comprises fibrinogen and a protease inhibitor,

- component B comprises a proteolytic enzyme being capable of cleaving specifically fibrinogen and causing formation of the fibrin polymer and

- additionally ingredients for culturing embryonic cells of mamals in one or both of the components A and B.

Preferably, cryoprecipitate of whole blood or plasma is used as fibrinogen containing fraction of component A. Although, commercially available cryoprecipitate can be used. It can be advantageous to concentrate the cryoprecipitate between a factor 2 and 5.

Preferably, the cryoprecipitate is virus inactivated. A procedure for virus inactivation is, for example, described in PCT/EP 91/00503. The basic principle of this method is the treatment of the cryoprecipitate with special detergents and removing the detergent later on from the cryoprecipitate.

Preferably, the protease inhibitor present in component A is aprotinin and present in concentrations up to 10,000 U/ml based on total volume of component A. Aprotinin is commercially available under the trademark Trasylol^{R} or Antagosan^{R}. Also tranexamic acid [4-(aminomethyl)cyclohexane carboxylic acid] or its acceptable salts is a suitable agent which can be used instead of aprotinin or in combination with.

The second component B of the two component fibrin-glue composition of the invention is prepared by solution of proteolytic enzyme being capable of cleaving specifically fibrinogen. Preferably, thrombin has been used which was isolated from plasma of human beings or mamals such as bovine. The thrombin can be delivered in a lyophilized form. The reconstitution of thrombin occurs with a solution containing calcium chloride. The concentration of the protease specifically for the cleavage of fibrinogen depends on the method of transferring the embryo into the uterus. Basically, if a slow working two component fibrin-glue composition is desired the concentration of thrombin should be lower whereas if a fast working fibrin glue is desirable the concentration of thrombin should be higher (fast fibrin-glue). Typically the concentration range of thrombin is 0.4 to 4 units/ml in a slow working fibrin-glue.

Another preferred embodiment of the fibrin-glue of the present invention comprises as component B a proteolytic enzyme which is isolated from snake venom. The snake venom enzyme batroxobin which can be isolated from the south american pit viper Bothrpos moujini can be used. Chemically this venom is a single chain glyco peptide with a molecular weight of approximately 36,000. It is known under the name Defibrase^{R} which causes cleavage of the ala-16-arg/17 glue bond in fibrinogen which causes the release of fibrino peptide A and the formation of monomeric fibrin I.

The use of any venom converting fibrinogen to fibrin such as Defibrase or Reptilase is preferred when the cryo-precipitate is made from autologous source and the use of the venom will avoid the use of human blood product.

According to the invention there must be present ingredients for culturing embryonic cells of mamals in the fibrin-glue of the invention. This can be achieved either by admixing the ingredients into one or both of the components A and B of the invention or dissolve these ingredients in a liquid and mixing this liquid to one or both of components A and B. Preferred ingredients for culturing embryonic cells of mamals are those comprising calcium chloride, potassium chloride, magnesium sulfate, sodium chloride, sodium bi-carbonate, sodium dihydrogenphosphate, D-glucose as well as phenol. Preferably, the solution has a pH of about 7.2. It can be advantageous to add additionally pyruvic acid and antibiotics, e. g. gentamycin.

A typical procedure for preparing component A in a virus free preparation is suggested in PCT/EP 91/01850.

In a typical procedure the cryoprecipitate and the thrombin is dissolved in the medium for culturing the embryo. Of course, both components are separated in order to prevent starting of the clot reaction. The composition of two preferred culture mediums are given below. The first one is the so-called EBSS which is commercially available from Gibco Ltd. EBSS is the abbreviation for Earle's Balanced Salt Solutions. It can be purchased in liquid or powder form. The liquid contains anhydrous calcium chloride in amounts of 0.2 g/l, potassium chloride 0.4 g/l, $MgSO_4$ x 7 H_2O 0.2 g/l, sodium chloride 6.8 g/l, sodium bicarbonate 2.2 g/l, NaH_2PO_4 x $2H_2O$ 0.158 g/l and additionally D-glucose 1.0 g/l and phenol red 0.01 g/l. The respective powder form contains after being dissolved in one liter 0.20 g/L calcium chloride anhydrous, 0.4 g/l sodium chloride, 0.0977 g/l magnesium

sulfate anhydrous, sodium chloride 6.80 g/l, $NaH_2PO_4 \times H_2O$ and the same D-glucose and phenol red content as in the liquid form. It can be advantageous to add pyruvic acid preferably in amounts of 0.015 g/l and gentamycin in amounts of 50 mg/l.

Depending on the field where the in vitro fertilization is carried out the medium can be modified in order to meet the special requirements which may be differ, for example, with respect with agricultural applications such as breeding. The above mentioned culture medium can be used also in human in vitro fertilization.

The advantage of the present fibrin-glue over the known fibrin-glue products having failed to improve the outcome of in vitro fertilization may be due to its increased anti-fibrinolytic activity preventing or postponing the embryo from one hand in the uterus on the other hand to degredate the clot. The two component fibrin-glue composition of the present invention enables the embryo to survive in the environment of the uterus before it is lodged and permenently fixed to the uterus wall. The surviving is supported by the medium containing ingredients for embryo cell culturing. Surprisingly, the high amounts of protease inhibitor like aprotinin do not interfere with the embryo but prevent the digest of the glue. Moreover, in another preferred embodiment of the present invention the two component fibrin-glue composition is iso-osmolar. Therefore, the disadvantage of the glues of the prior art being hyper-osmolar is prevented. Hyper-osmolarity causes the death of the embryo by drying out phenomena.

The advantageous features of the fibrin-glue of the invention are the presence of salts and nutrients which are important for in vitro growing of the embryo, it contains anti-fibrinolytic activity to postpone the early degradation of the glue either by embryo or by the uterus. Moreover, the

antifibrinolytic potency of the embryo has to be balanced to the extent that it will enable the fetus to lyse a certain area around itself creating a halo like shape of fluid surrounded by the fibrin-glue.

Under certain circumstances it could be recommendable to include a growth factor that will enhance and speed up the maturation of the fetus.

The use of cryoprecipitate as a source of fibrinogen rather than a fibrinogen concentrate is advantageous since the former one contains ingredients like fibronectin, von Willebrand factor etc. which may be important for adhesion. Moreover, a cryoprecipitate may also contain other agents such as proteins or low molecular substances which support the development and lodging of the embryo. Also compounds having wound healing properties such as hyaluronic acid can be used.

For preparing the two component fibrin-glue composition of the invention (as it can be used in the experiments as described above) cryoprecipitate is dissolved in culture medium as described above. Then aprotinin or tranexamic acid is added to the cryoprecipitate. The concentration of aprotinin in the cryoprecipitate containing component A is preferably in the range of 6,000 to 10,000 u/ml. The concentration of tranexamic acid is preferably 10 - 200 µg/ml (final concentration). This is equivalent to an aprotinin activity of about 3,000 to 10,000 KIU/ml. The final concentration of aprotinin in the mixture of component A and B is lower because of the dilution when component B is added. The dilution factor depends on the amount of component B which is added. The concentration of fibrinogen in the cryoprecipitate solution is relatively low compared with other fibrin-glues since there is no need for strong tensile strength. Preferred are concentrations of fibrinogen in the range of 5 to 20 g/l.

The embryo is injected for example via the following method. The embryo is placed in the reconstituted cryoprecipitate solution in a culture dish. Then thrombin is added in concentrations of 0.4 to 4 units. The thrombin concentration determines the clotting time of the glue. the embryo is sucked in the culture fluid and injected into the uterus.

Claims

1. A two component fibrin-glue composition comprising the components A and B wherein

   - component A comprises fibrinogen and a protease inhibitor,

   - component B comprises a proteolytic enzyme being capable of cleaving specifically fibrinogen and causing formation of the fibrin polymer and

   - additionally ingredients for culturing embryonic cells of mamals in one or both of the components A and B.

2. The composition of claim 1 wherein the fibrinogen containing a fraction of component A is cryoprecipitate of whole blood.

3. The composition of claims 1 and/or 2 wherein the protease inhibitor of component A is aprotinin in amounts up to 10,000 u/ml based on total volume of component A and/or 4-(aminomethyl)cyclohexane carboxylic acid or its pharmaceutically acceptable salts.

4. The composition of any one of the claims 1 to 3 wherein the proteolytic enzyme of component B is thrombin or a proteolytic enzyme derived from snake venom such as batroxobin isolated from venom of the south american pit viper Bothrpos moujini.

5. The composition of any one of the claims 1 to 4 wherein component A is virus inactivated cryoprecipitate.

6. The composition of any one of the claims 1 to 5 wherein the culture medium for growing embryonic cells contains calcium chloride, potassium chloride, magnesium sulfate, sodium choride, sodium bicarbonate, sodium dihydrogenphosphate, D-glucose as well as phenol red at a pH of about 7.2 and containing optionally pyruvic acid and/or gentamycin.

7. The composition of any one of the claims 1 to 6 having an agent with wound healing such as hyaluronic acid.

8. Method of improving the success of in vitro fertilization by attaching an embryo to the endometrium whereby the embryo is transferred together with the two component fibrin-glue composition of any one of the claims 1 to 6 into the uterus.

9. Method according to claim 8 for in vitro fertilization in breeding of livestock or cattle breeding.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 93/01797

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61L25/00; A61K35/54

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61L ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	EP,A,0 339 607 (S. ITAY) 2 November 1989 see column 4, line 15 - line 52; claims 1,4; examples 1-4	1-3
P,X	WO,A,9 222 312 (WADSTRÖM J.) 23 December 1992 see page 1, line 37 - line 40 see page 3, line 34 - line 39 see page 4, line 1 - line 10 see page 7, line 1 - line 17 see page 9, line 14 - line 20; examples 1-5	1-3,7
P,A	WO,A,9 215 341 (PENTAPHARM) 17 September 1992 see claims 1-3,10	4

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° Special categories of cited documents :[10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
06 OCTOBER 1993	10. 93
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	GB,A,2 102 811 (IMMUNO) 9 February 1983 see page 1, line 45 - line 55; examples 1-4	1-3

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

EP 9301797
SA 76739

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
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